



# Contrasting seasonal morphological and physio-biochemical responses to UV radiation and reduced rainfall of two mature naturally growing Mediterranean shrubs in the context of climate change

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## ABSTRACT

Naturally growing *Arbutus unedo* L. and *Phillyrea angustifolia* L. mature shrubs with similar leaf sclerophyllous index were studied. The aim was to evaluate whether: 1) ultraviolet radiation (UV) effects on plant development differ depending on the type of UV radiation (UV-A or UV-B), 2) rainfall amount modulates plant responses to UV and 3) seasonal climatic conditions influence the response of plants to the UV and/or rainfall treatments applied. To achieve these goals, *A. unedo* and *P. angustifolia* plants were subjected to UV-B exclusion (UVA), UV-B + UV-A exclusion (UV0) or near-ambient UV (UVBA) levels, in combination with two rainfall conditions (natural rainfall or rainfall reduction). Leaf morphological (leaf mass per area, leaf density, thickness and area), physiological (leaf relative water content, gas exchange and chlorophyll fluorescence) and biochemical (leaf C and N content, and leaf concentration of photosynthetic pigments and phenols) parameters were analysed seasonally for a year. Effects of UV and rainfall treatments on most of the parameters analysed in both species did not depend on the season. In any of the two species, morphological parameters responded to the treatments, likely because of the high leaf mass per area of the sampled fully-developed leaves. In *A. unedo*, neither physiological nor biochemical leaf traits varied significantly among UV conditions, probably due to their intrinsic high leaf concentration of phenols, which could also explain the lack of correlation between leaf traits and leaf carbon content. Conversely, leaf nitrogen content was highly correlated with leaf features when *A. unedo* plants were grown exposed only to UV-A radiation. Overall, *A. unedo* would be less responsive to UV, but not to a decrease in water availability, with plants displaying a water-spender strategy under reduced rainfall. In contrast, *P. angustifolia* responded at physiological level to the combination of both treatments. Indeed, plants subjected to low rainfall and only UV-A showed lower leaf transpiration rates and stomatal conductance than UV0 plants, higher leaf electron transport rates than UVBA plants, and the highest leaf water use efficiency. These results suggest that, under reduced rainfall, plant exposure only to UV-A would trigger the water-saving response typical of *P. angustifolia*, despite the mild reduction (20%) in soil water content applied. Under natural conditions, this UV-A-induced response of plants to mild water deficit seems to be masked by the presence of UV-B radiation.

## 1. Introduction

Climatic models predict that, in the next years, Mediterranean plants will be subjected to an increase in ultraviolet radiation (UV) levels (Bais et al., 2015) and a decrease in water availability, mainly in summer, as a consequence of a reduction in mean cloudiness (Sanchez-Lorenzo et al., 2017). UV reaching the Earth's surface comprises UV-B (280–315 nm) and UV-A (315–400 nm), which represent 1.5% and

6.5%, respectively, of the sun electromagnetic radiation. UV-B is mostly blocked by the ozonosphere being more energetic than UV-A, but UV-A accounts for up to 95% of the UV reaching the ground (Moan, 2001) being able to penetrate deeper in plant tissues (Liakoura et al., 2003). From the nineties many studies have focused on elucidating the effects of an increase in UV-B on plant development (for reviews, see Björn et al., 1997; Ballaré et al., 2011; Jansen and Bornman, 2012; Bandurska et al., 2013). Conversely, despite UV-A is also expected to increase in

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the near future in the Mediterranean basin, there is very few information available on the effects of this type of UV on plant growth and development, being most studies conducted in crop species (for a review, see Verdaguer et al., 2017).

Most researchers agree that high UV-B levels exert a genotoxic effect (Jansen and Greenberg, 1998; Hollósy, 2002; Zlatev et al., 2012), while low UV-B levels would act as an environmental cue regulating a wide range of developmental processes, mostly through the activation of the UVR8 photoreceptor (Hideg et al., 2013; Morales et al., 2013; Jenkins, 2014). For instance, the increase in UV-B would promote the growth of plants with a more compact architecture and smaller and thicker leaves, which would decrease the intercepted UV and, thus, would reduce damage to the photosynthetic tissues (Robson et al., 2015). Enhanced UV-B has also been linked to an increase of leaf phenolic compounds, either for their UV-screening or their antioxidant capacity (Searles et al., 2001; Hideg et al., 2013; Nenadis et al., 2015). However, many UV-B-sensitive species show a reduction in plant biomass accumulation mostly related to a UV-B-impairment of photosynthetic processes (Teramura and Sullivan, 1994; Feng et al., 2003; Kataria et al., 2013). In relation to UV-A, effects are strongly species-specific and often distinct from those triggered by UV-B, which imply that distinct action mechanisms would be elicited by different UV wavelengths (Verdaguer et al., 2017). Indeed, in some species, UV-B reduced plant growth while UV-A increased it (Tezuka et al., 1993; Cooley et al., 2001; Kataria et al., 2013). Plant growth stimulation in response to UV-A has been related to an increase in photosynthesis, likely associated to higher leaf chlorophyll content (Tezuka et al., 1993; Tezuka et al., 1994), an increase in stomatal opening due to the activation of UV-A-sensitive photoreceptors (Araújo et al., 2011; Aggarwal et al., 2014), or an increase in the photoprotection of the photosynthetic apparatus (Joshi et al., 2007; Bernal et al., 2015). Nevertheless, UV-A-induced plant growth reductions have also been reported (Newsham et al., 1999; Cooley et al., 2001; Kataria et al., 2013), despite photosynthetic activity increased (Kataria et al., 2013), being still a matter of concern the elucidation of the mechanisms triggered by UV-A, especially in woody species (Verdaguer et al., 2017).

Mediterranean evergreen sclerophyllous species will probably need to activate a variety of response mechanisms to tolerate and survive to an enhancement of UV levels in combination with a reduction in water availability, which can significantly differ from those exhibited in response to each one of the factors separately (Mittler, 2006). In the context of climate change, diverse studies have been made about the combined effects on plants of UV-B and other abiotic factors, such as CO<sub>2</sub>, nutrients or precipitation (Ballaré et al., 2011). Regarding the latter, some studies suggest a cross-tolerance effect between UV-B and water deficit, becoming UV-B-treated plants more tolerant to water stress or *vice versa* (Bandurska et al., 2013). These responses, reported mainly in species of agricultural interest, are achieved either by modifying leaf morphology (Murali and Teramura, 1986; Balakumar et al., 1993; Feng et al., 2007), by enhancing photosynthesis and/or leaf water status (Balakumar et al., 1993; Alexieva et al., 2001; Poulson et al., 2002; Feng et al., 2007; Cechin et al., 2008), or by varying the levels of some metabolites (Schmidt et al., 2000; Alexieva et al., 2001; Poulson et al., 2006) such as the UV absorbing compounds (Murali and Teramura, 1986; Noguez et al., 1998). In woody species, much less information is available, and while, in some species, drought would contribute to mask UV-B effects (Duan et al., 2008) or UV-B would alleviate summer water scarcity (Björn et al., 1997; Drilias et al., 1997; Manetas et al., 1997), no interactive effects were found in other species (Nogués and Baker, 2000; Kyparissis et al., 2001; Bernal et al., 2013). To our knowledge, there are even fewer data regarding the combined effects on plants of enhanced UV-A and drought. Recently, our group has published two studies using seedlings from Mediterranean woody species in which UV-A seems to exert a positive effect on root biomass accumulation (Bernal et al., 2013) or on total plant growth (Bernal et al., 2015) under low water availability. This effect was related to a

UV-A-induced increase in leaf photosynthetic rates and/or water use efficiency (WUE) under low watering (Bernal et al., 2015), but more studies are needed to disentangle this question.

Most of the previous studies aimed to provide information about the putative effects of UV and drought on plants have been performed using potted seedlings, even in the case of shrubs or trees. From seedlings to mature trees, plants are subjected to a profound structural change, decreasing photosynthesis and stem hydraulic conductance, but increasing the root system providing plant access to deeper water soil layers (Niinemets, 2010; Sperlich et al., 2015). Moreover, a shift from resource-acquisitive to resource-conservative strategies concomitantly with leaf (Mason et al., 2013) and plant ontogeny (Niinemets, 2006) has been reported. Unlike seedlings, older evergreen Mediterranean sclerophyllous plants are likely adapted to cope with the repeatedly adverse environmental conditions experienced on summer and winter days (Oliveira and Peñuelas, 2000; Llorens et al., 2003; Gratani et al., 2016). Thus, in general, it is considered that seedlings are more sensitive to stress events than mature plants (Niinemets, 2010; Sperlich et al., 2015), which imply that scaling responses from seedlings to mature trees or shrubs can result in overestimated conclusions (Niinemets, 2006). In this sense, as far as we know, there are no studies on the effects of UV-B and UV-A radiation, in combination with low water availability, on the morphology and physio-biochemical features of naturally growing woody plants.

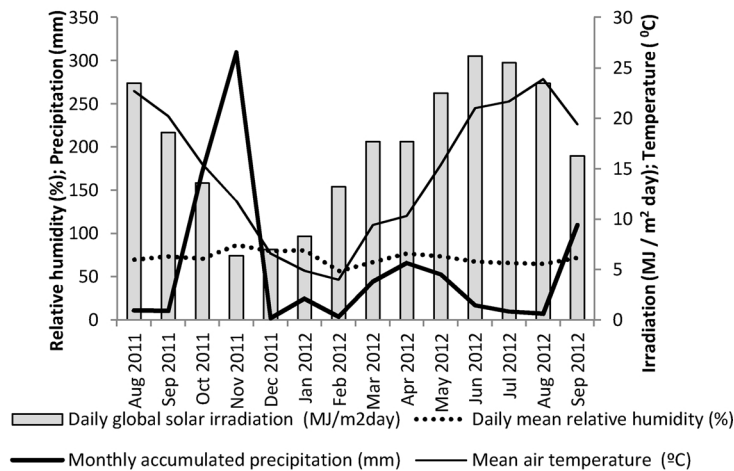
Therefore, the current study was designed to test the hypotheses that, in mature naturally growing Mediterranean evergreen species, UV-A and UV-B effects on leaf morphology, physiology and biochemistry mostly differ, and that water shortage modulate these responses, being plants more sensitive to UV under summer climatic conditions, i.e. when UV is highest and drought is more acute. To test these hypotheses, a field experiment was performed in order to expose mature naturally growing *Arbutus unedo* L. and *Phillyrea angustifolia* L. plants to three UV conditions (UV-B + UV-A exclusion, UV-B exclusion, and ambient UV-B + UV-A) in combination with two rainfall regimes (natural and reduced rainfall). In both species, leaf morphological (leaf mass per area, leaf density, thickness and area), physiological (leaf relative water content, gas exchange and chlorophyll fluorescence) and biochemical (leaf content of photosynthetic pigments, phenols, C and N) parameters were analysed seasonally. *A. unedo* and *P. angustifolia* are two of the dominant species of the studied Mediterranean shrubland, *Lavandulo-Ericetum scopariae* Br.Bl. 1931 (Braun-Blanquet, 1932) community. Both species are evergreen sclerophyllous with a leaf mass per area (LMA) around 15 mg cm<sup>-2</sup>, which is common for Mediterranean woody plants and indicative of a leaf conservative resource-use strategy (Poorter et al., 2009; Domínguez et al., 2012). Moreover, both species have high ecological interest, since they are a source of food for animals (Döring and Hoffmann, 2004; Rosalino et al., 2010) and have a great capacity to resprout after fire which favours the regeneration of the ecosystem (Paula et al., 2016).

## 2. Material and methods

### 2.1. Study area, experimental design and plant species

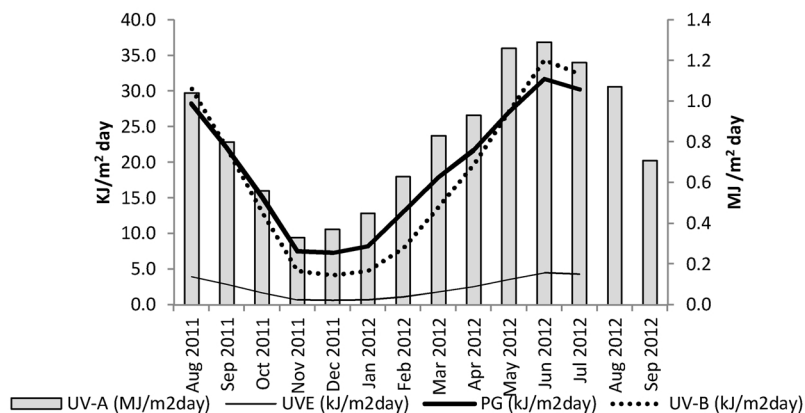
In August 2011, an outdoor experiment was started in a forested naturally-growing Mediterranean shrub community (Gavarres Massif, 41° 53' N, 2° 54' E; Cassà de la Selva, NE of the Iberian Peninsula) established for a minimum of 70 years and subjected to natural disturbances, such as droughts, fires or grazing. The experiment was placed on a south-facing slope at 250 m above sea level in a community dominated by *Arbutus unedo* L., *Phillyrea angustifolia* L. and *Erica scoparia* L., whose relative abundances were around 12%, 17% and 36% respectively. A few specimens of cork-oak (*Quercus suber* L.) and pines (*Pinus pinaster* Aiton and *P. pinea* L.), and some of *Calluna vulgaris* (L.) Hull, *Viburnum tinus* L., *Daphne genkium* L., *Ulex parviflorus* Pourr. and *Cistus salviifolius* L. and the herbaceous *Brachypodium retusum* and *Carex*

A



**Fig. 1.** A) Monthly means of daily global solar irradiation ( $\text{MJ}/\text{m}^2\text{day}$ ), air temperature ( $^{\circ}\text{C}$ ), daily relative humidity (%) and monthly accumulated precipitation, and B) monthly means of unweighted UV-A ( $\text{MJ}/\text{m}^2\text{day}$ ) and UV-B ( $\text{KJ}/\text{m}^2\text{day}$ ) radiation, and the ground-level ultraviolet levels weighted with the erythral irradiance algorithm (UVE,  $\text{KJ}/\text{m}^2\text{day}$ ) and the generalized plant growth weighting function (PG,  $\text{KJ}/\text{m}^2\text{day}$ ).

B



*oedipostyla* were also present in the studied community. The soil was mainly siliceous with very good drainage (Díaz-Guerra et al., in press).

The experimental setup has been described previously in Nenadis et al. (2015). The experiment was arranged in a randomized block design with treatments (UV radiation and rainfall) being replicated 3 times. Briefly, 18 plots were distributed in 3 blocks of 6 plots over the study area, each block having 2 plots of each one of 3 UV radiation conditions, one for each rainfall level (see rainfall treatment description below). Each plot had an area of  $9\text{ m}^2$  ( $3\text{ m} \times 3\text{ m}$ ) and was covered with roofs of plastic filters suspended over metal frames. At the centre of the plots, the roofs were at a height of 155 cm from the soil, which allowed ventilation. The roofs sloped down  $10^{\circ}$  towards the south and direct solar radiation from the south face was minimised by means of a 40 cm-wide fringe made from the same material as the roof. At the south face, natural rainfall was collected with a gutter and stored in a tank (310 L of capacity).

At the beginning of the study, experimental plots had a vegetation cover of 45–89% and each plot had two or three individuals of *A. unedo*, and from three to six *P. angustifolia* plants. The initial size of plants from both species growing in the plots was determined. Specifically, maximum height was 40–117 cm and 46–125 cm and maximum diameter 41–132 cm and 42–128 cm for *A. unedo* and *P. angustifolia* plants, respectively. Moreover, both species were multi-stemmed, having *A. unedo* and *P. angustifolia*, respectively, 3 to 72 and 6 to 43 stems per plant.

Meteorological data (daily global solar irradiation, rainfall, air temperature and relative humidity) were obtained from August 2011 to

September 2012 from the weather station of Cassà de la Selva, 3 km away from the experimental site (177 m above sea level,  $41^{\circ} 52' \text{ N}$ ,  $2^{\circ} 55' \text{ E}$ ) (Fig. 1A).

## 2.2. UV reduction treatment

To study the UV reduction effect on plant growth, three different conditions were created by using different filters as roofs of the experimental plots. Hence, near-ambient solar UV was provided in 6 plots (hereafter UVBA plots), 2 per block, by means of a 3 mm-thick methacrylate cover that transmitted the UV from 280 to 400 nm (MC0100XN, Polimer Tecnic, Girona, Spain); in 6 other plots (hereafter UVA plots), strong reduction of the UV-B (280–320 nm) was achieved by means of a 0.25 mm-thick polyester cover (Melinex, Ponscosta, Valencia, Spain); and finally, in the last 6 plots (hereafter UV0 plots), almost all the solar UV-A and UV-B was excluded using a 2 mm-thick polycarbonate cover (PC0100UV, Polimer Tecnic, Girona, Spain). Methacrylate and polycarbonate filters were often cleaned, whereas polyester filters were periodically replaced.

The transmittance of the cover filters was measured in the UV and visible bands in the laboratory by means of a deuterium/halogen lamp and a CCD spectrophotometer (Avantes, The Netherlands). In the field, at least in one campaign each season and during sunny days at noon, spectral irradiances covering the UV and photosynthetically active radiation (PAR) fluxes were measured under the filters with a double monochromator spectroradiometer (SR9910, Irradian Ltd. UK), and compared with the values outside the covers (Nenadis et al., 2015). The

retrieved photosynthetic photon flux density (PPFD), and unweighted UV-B and UV-A irradiances were, respectively, around 84%, 5% and 6% of ambient in UV0 plots, 85%, 3% and 52% of ambient in UVA plots, and 91%, 81% and 85% of ambient in UVBA plots. Weighted UV irradiances according to the Plant Growth (PG) response spectrum proposed by (Flint and Caldwell, 2003), were 84%, 41% and 5% in UVBA, UVA and UV0 plots, respectively. In addition, to assess monthly UV levels, erythemal UV irradiance (UVE) was measured with a UV-S-E-T Kipp & Zonen sensors (The Netherlands) placed at the radiometric station of the Environmental Physics Group (EPG) at the University of Girona (41.972°N, 2.829°E, 115 m a.s.l.). The UVE and PPFD data in combination with the spectral measurements and modelling allowed the estimation of unweighted and weighted UV irradiances (Fig. 1B).

### 2.3. Rainfall treatment

Two rainfall levels were applied, with half of the plots being watered with all the natural rainfall (hereafter NR plots) and the other half receiving 70% (in spring, summer and autumn) or 90% (in winter) of the natural rainfall (hereafter RR plots). The percentage of rainfall reduction was established according to the forecasted changes for the Mediterranean basin (IPCC, 2013). For each plot, natural rainfall was collected with a gutter, stored in a tank (310 L of capacity) and, after each rainfall event, supplied to the plots with a pump and a hose, according to its rainfall condition (Table S1). Soil moisture, measured as the percentage of volumetric water content at the topsoil (0–20 cm depth) using a time domain reflectometer (Field Scout TDR300, Spectrum Technologies, Inc. USA), was significantly lower (20%) in RR plots compared to NR plots over the study period (Fig. S1). Within each UV condition, soil moisture was also lower in RR than in NR plots; concretely, it was 29%, 20% and 10% lower in UV0, UVA and UVBA plots, respectively (Fig. S1).

### 2.4. Leaf sampling

Samplings were conducted at the end of four consecutive seasons: autumn 2011, and winter, spring and summer 2012 (specifically, the sampling campaigns were on: 12–22 December 2011, 8–15 March 2012, 11–21 June 2012 and 11–20 September 2012), always at midday under clear sky conditions. Leaves were always harvested from different stems of one or two *A. unedo* plants, and from a minimum of three randomly chosen *P. angustifolia* plants. Leaves selected were at most one year old, being always fully expanded, south oriented and located at the top of the plant canopy.

### 2.5. Leaf morphological parameters and relative water content

Four leaves for *A. unedo* and three for *P. angustifolia*, were sampled per plot and season to determine leaf area, thickness, relative water content (RWC, %), mass per area (LMA,  $\text{mg cm}^{-2}$ ) and density (LD,  $\text{mg cm}^{-3}$ ). In the field, sampled leaves were stored in a cool-box and then carried to the lab where measurements were immediately conducted. Foliar thickness was measured with a portable micrometer (mod. 4000DIG, Baxlo, Spain). Then, leaves were scanned (Epson perfection 1250, USA), and leaf area was determined using an image-analysis programme (ImageTool, University of Texas Health Science Center, USA). To calculate leaf RWC, leaves were weighed after collection to obtain their fresh mass (FM) and then they were kept in distilled water in darkness in the fridge at 4 °C for 24 h to determine their turgid mass (TM). After that, leaves were oven dried at 45 °C for 5 days and the dry mass (DM) was obtained. RWC was calculated as:  $(\text{FM}-\text{DM})/(\text{TM}-\text{DM}) \times 100$ . Finally, LMA was determined as the ratio between DM and area for each leaf, and leaf density was estimated by dividing LMA by thickness (Witkowski and Lamont, 1991).

### 2.6. Leaf gas-exchange parameters

Foliar gas exchange parameters, including transpiration rates (E), stomatal conductance ( $g_s$ ) and net photosynthetic rates (A), were measured in four and three attached leaves in *A. unedo* and *P. angustifolia*, respectively, per plot and season. These parameters were determined using a portable open-circuit infrared gas analyser system (CIRAS-2, PP-Systems Inc. Amesbury, USA) at about 400 ppm of  $\text{CO}_2$ . Since leaves of *P. angustifolia* were narrow and did not fill completely the chamber of the leaf cuvette (PLC6–18 mm of diameter; PP-systems Inc, Amesbury, USA), gas-exchange parameters were recalculated considering only the fraction of the leaf area in which measurements were taken. To do this, the leaf fractions used for the measurements were cut in the field, stored in a cool-box and carried to the lab, where they were scanned to determine their area by means of an image-analysis programme (ImageTool). Intrinsic water use efficiency was calculated as  $A/g_s$  (WUE).

### 2.7. Leaf chlorophyll fluorescence parameters

Modulated chlorophyll fluorescence of the adaxial surface of attached leaves was measured at midday using a portable modulated fluorimeter (PAM-2100 Heinz Walz GmbH, Effeltrich, Germany) in four and three leaves of *A. unedo* and *P. angustifolia*, respectively, per plot and season. The following parameters, described previously in Bernal et al. (2015), were measured: the potential photochemical efficiency of photosystem II (PSII) or  $F_v/F_m$ , where  $F_v$  is the variable fluorescence calculated as  $F_v = F_m - F_0$ , being  $F_0$  the minimum and  $F_m$  the maximum dark-adapted (30 min) fluorescence; the actual photochemical efficiency of PSII in the light-adapted state or  $\Delta F/F_m' = (F_m' - F)/F_m'$ , where  $F$  is the steady-state fluorescence yield under the given environmental conditions and  $F_m'$  is the maximum level of fluorescence obtained during a saturating flash of light; the apparent electron transport rate or  $\text{ETR} = \Delta F/F_m' \times \text{PAR} \times 0.84 \times 0.5$ , where PAR was expressed in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 0.84 was the assumed coefficient of absorption of the leaves, and 0.5 was the assumed distribution of absorbed energy between the two photosystems (Galmés et al., 2007), and finally the non-photochemical quenching coefficient determined as  $\text{NPQ} = (F_m - F_m')/F_m'$ .

### 2.8. Leaf photosynthetic pigment content

Twelve foliar discs from four leaves (three discs per leaf) of *A. unedo*, and from six to eight whole leaves of *P. angustifolia* were sampled per plot in winter and summer campaigns. Samples were immediately frozen in liquid nitrogen in the field, being carried to the lab where they were stored at  $-80^\circ\text{C}$  until analysis. Photosynthetic pigments were extracted from three foliar discs (*A. unedo*) or from two leaf fractions (*P. angustifolia*) randomly taken from each composite sample. The leaf tissue was homogenized in a mortar with acetone and 0.01 g of  $\text{CaCO}_3$  and the extract was filtered through a syringe with a fiberglass filter (AP2001300, Millipore, Ireland). Finally, the absorbance was read with a spectrophotometer (Genesys 6, Thermo electron corporation, USA) at 663.6, 646.6 and 470 nm. The concentrations of chlorophylls (Chl) were calculated applying the following equations:  $\text{Chl } a$  ( $\mu\text{g/ml}$ ) =  $12.25 (A_{663.6}) - 2.55 (A_{646.6})$ ,  $\text{Chl } b$  ( $\mu\text{g/ml}$ ) =  $20.31 (A_{646.6}) - 4.91 (A_{663.6})$ , and  $\text{Chl } a + b$  ( $\mu\text{g/ml}$ ) =  $17.76 (A_{646.6}) + 7.34 (A_{663.6})$  (Porra, 2002), and the leaf concentration of carotenoids was determined as:  $\text{Car} (\mu\text{g/ml}) = (1000 (A_{470}) - 3.27 [\text{Chl } a] - 104 [\text{Chl } b])/227$  (Lichtenthaler and Wellburn, 1983). The content of pigments was, then, expressed as  $\text{mg g}^{-1}$  dry weight (DW), and the ratios  $\text{Chl } a/\text{Chl } b$  and  $\text{Car}/\text{Chl } a + b$  were calculated.

### 2.9. Leaf phenolic content

The Folin-Ciocalteu method was applied as described in Bernal et al. (2013) to determine the leaf total phenolic content in the same A.



*unedo* and *P. angustifolia* leaves that RWC was measured in winter and summer campaigns. Specifically, 4–6 mg of dry material randomly taken from each sample was grounded with a ball mix (Mixer Mill MM400, Retsch, Germany) at 30 Hz for 3 min. The powder was mixed with 2.5 mL of 50% methanol, being the extract shaken for 1 h and centrifuged for 5 min at 2500 rpm. A fraction of the extract (50  $\mu$ L) was mixed with 3.5 mL of distilled water and 250  $\mu$ L of Folin-Ciocalteu reagent (Merck, Germany). After 8 min, 750  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> (20%) was added. Absorbance was measured 2 h later at 760 nm with the spectrophotometer. The total phenolic content of leaves was calculated from the standard curve for gallic acid prepared using concentrations from 40 to 1000 mg L<sup>-1</sup> and expressed as gallic acid equivalents (GAE, mg g<sup>-1</sup> DW).

## 2.10. Leaf carbon and nitrogen content

Four leaves of *A. unedo* and three of *P. angustifolia* per plot and season were separately oven dried at 45 °C for 5 days and grounded using a ball mill (Mixer Mill MM400, Retsch, Germany). For each sample, 3–4 mg of powder was placed in tin capsules and send to the Stable Isotope Facility at the University of California (Davis, USA) to analyse the leaf carbon and nitrogen content using an elemental analyser (PDZ Europa ANCA-GSL, Sercon Ltd., Cheshire, UK) linked to a continuous flow isotope ratio mass spectrometer (IRMS; PDZ Europa 20-20 IRMS, Sercon Ltd., Cheshire, UK).

## 2.11. Statistical analyses

Statistical analyses were conducted separately for both species applying different Analysis of Variance (ANOVA). In the case of *A. unedo*, because the same individuals were followed throughout the whole studied period (four seasons), measured data were analysed by using a two-way repeated-measures ANOVAs (with UV and rainfall treatments as fixed factors). One mean per plot, obtained by averaging the four data taken for each variable from one or two plants was used for these analyses. When season was significant, one-way ANOVA and subsequent post hoc comparisons (Tukey's HSD tests) were applied. In the case of *P. angustifolia*, since plants were randomly chosen (plots had more than three plants), four-way ANOVAs were performed for all variables except for the leaf photosynthetic pigments' content, with season, UV treatment and rainfall regime as fixed factors and block as a random factor. The amount of leaf pigments were analysed by means of three-way ANOVAs, since a composite sample was collected per plot. For both species, when significant interactions among fixed factors were found, we performed ANOVAs to determine the effects on the studied variables of one treatment within the levels of the other treatment or within each season. Kolmogorov-Smirnov and Levene's tests were used to examine normality and homoscedasticity, respectively, and data were log-transformed when necessary. Specifically, (log (x + 10)) was used to transform leaf area, E, g<sub>s</sub> and F<sub>v</sub>/F<sub>m</sub> for *P. angustifolia*; while, in the case of *A. unedo*, both square root ( $\sqrt{x}$ ; for WUE) and logarithm (log(x + 10); for F<sub>v</sub>/F<sub>m</sub>) were used. When normally distributed data did not meet the assumption of homoscedasticity, the Games-Howell post-hoc test was applied. To determine whether the filter *per se* could have an effect on the studied parameters, we statistically compared, for each species, and for those parameters responsive to the UV and/or rainfall treatment (Table 1), data from individuals growing in control plots (UVBA plots under natural rainfall) with data from similar individuals growing outside the plots (Table S2). The relationship between measured variables and leaf carbon and nitrogen content were analysed by means of Pearson's correlation tests for each species separately and within each UV condition (Table 2). Differences were considered statistically significant at  $p \leq 0.05$ , except in the case of Pearson's correlations where  $p \leq 0.025$  was considered as the level of significance. Statistical analyses were conducted using SPSS Statistics packages (IBM; USA).

## 3. Results

### 3.1. Environmental data

The meteorological data measured during the study period for the four seasons are shown in Fig. 1. The monthly means of daily relative humidity, accumulated precipitation and air temperature were the typical of an area subjected to the Mediterranean climate characterised by cool, dry winters and hot, dry summers (Fig. 1A). The monthly means of daily global solar irradiation (Fig. 1A) and of unweighted UV-A (MJ/m<sup>2</sup>day) and UV-B (KJ/m<sup>2</sup>day) radiation followed the expected seasonal fluctuations for the Mediterranean climate (Fig. 1B). The maximum of daily global solar irradiation was reached in June and was around 26 MJ/m<sup>2</sup> and the UV-B and UV-A maximum reached 33 KJ/m<sup>2</sup> and 1.3 MJ/m<sup>2</sup>, respectively (Fig. 1B).

### 3.2. Treatment effects on *Arbutus unedo* L. leaf traits

Area, thickness, LMA, density and RWC of *A. unedo* leaves were not affected by the UV and/or rainfall treatments applied (Table 1). Conversely, plants of this species grown under reduced rainfall showed higher leaf stomatal conductance (g<sub>s</sub>), transpiration (E) and photosynthetic rates (A) (28%, 18%, 7%, respectively) than plants receiving natural rainfall (F<sub>1,16</sub> = 6.39,  $p = 0.02$ ; F<sub>1,16</sub> = 4.56,  $p = 0.05$ ; F<sub>1,16</sub> = 6.35,  $p = 0.02$ , respectively) (Table 1). Interestingly, when rainfall treatment effects on leaf gas exchange parameters were analysed within each UV condition, they were only significant for UVA plants. Indeed, plants grown exposed only to UV-A presented higher g<sub>s</sub> (57%), E (33%) and A (32%) (F<sub>1,19</sub> = 10.29,  $p = 0.01$ ; F<sub>1,19</sub> = 5.87,  $p = 0.03$ ; F<sub>1,19</sub> = 5.20,  $p = 0.03$  respectively) under reduced rainfall (Fig. 2A–C). Such increases did not affect the intrinsic water use efficiency (A/g<sub>s</sub>, WUE) of *A. unedo* leaves exposed to UV-A (Fig. 2D)

Parameters derived from foliar chlorophyll fluorescence measurements (ETR, F<sub>v</sub>/F<sub>m</sub> and NPQ), as well as the leaf content of chlorophylls and phenols, were not affected by the UV and/or the rainfall treatments (Table 1).

### 3.3. Treatment effects on *Phillyrea angustifolia* L. leaf traits

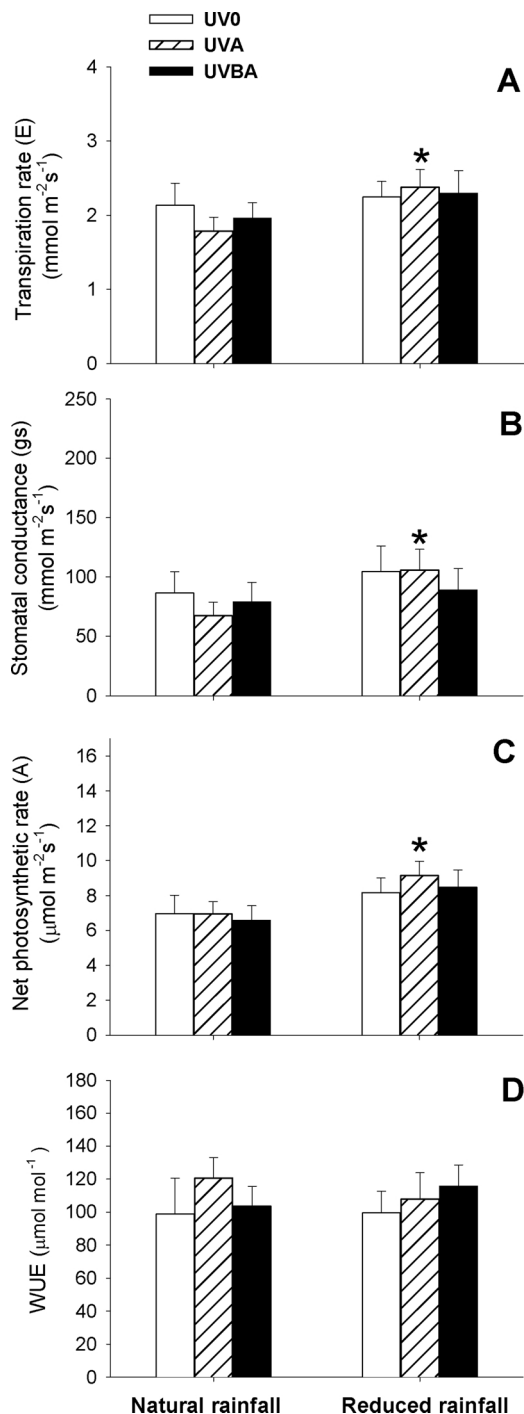
The UV and/or rainfall treatments did not affect *P. angustifolia* leaf morphological (area, thickness, LMA and density) or biochemical (leaf content of photosynthetic pigments and phenolics) parameters, while the studied physiological traits were more responsive to the treatments, being most of the UV effects modulated by water availability (Table 1). Indeed, an interactive effect between UV and rainfall was found for E (F<sub>2,213</sub> = 3.193;  $p = 0.04$ ), g<sub>s</sub> (F<sub>2,213</sub> = 2.93;  $p = 0.05$ ) and WUE (F<sub>2,213</sub> = 4.79;  $p = 0.01$ ) (Table 1). Under reduced precipitation, plant exposure only to UV-A led to lower E (by 26%) and g<sub>s</sub> (by 39%) (F<sub>2,84</sub> = 4.76,  $p = 0.01$  and F<sub>2,84</sub> = 3.46  $p = 0.04$ , respectively) compared to plants growing with almost no UV (UV0) (Fig. 3A and B). UV0 plants also showed higher E and g<sub>s</sub> values under reduced rainfall than when grown under natural rainfall (F<sub>1,52</sub> = 5.14,  $p = 0.03$  and F<sub>1,52</sub> = 6.03  $p = 0.02$ , respectively) (Fig. 3A and B). UV did never modify leaf A (Fig. 3C). Because of this, the significantly higher WUE values found under reduced precipitation in plants exposed only to UV-A (F<sub>2,84</sub> = 9.52,  $p < 0.001$ ) (36% and 42% higher than UV0 and UVBA plants, respectively) would be explained by their lower E and g<sub>s</sub> values (Fig. 3D). UVA plants also showed higher WUE values under reduced water availability than under natural precipitation (F<sub>1,62</sub> = 6.26,  $p = 0.04$ ) (Fig. 3D). UV did not affect foliar E, g<sub>s</sub> and WUE when plants received all the rainfall (Fig. 3A–D).

Analyses of chlorophyll fluorescence parameters revealed that UV effects on leaf ETR and F<sub>v</sub>/F<sub>m</sub> were dependent on the rainfall regime (F<sub>2,213</sub> = 4.34;  $p = 0.01$ ; F<sub>2,213</sub> = 4.21;  $p = 0.01$ , respectively; Table 1). Indeed, UV effects on foliar ETR were only significant for plants grown under reduced precipitation, while the opposite was found for F<sub>v</sub>/F<sub>m</sub>.

Table 1

Overall means  $\pm$  standard errors for the studied leaf morphological, physiological and biochemical parameters in *Arbutus unedo* L. and *Phillyrea angustifolia* L. plants grown under three UV radiation conditions (UV0, UVA and UVBA) and two rainfall regimes (natural and reduced rainfall). For *A. unedo* leaves, the sample size used was  $n = 18$  for each season,  $n = 24$  for each UV condition and  $n = 36$  for each rainfall regime. In the case of *P. angustifolia* leaves, the sample size used was  $n = 54$  for each season,  $n = 72$  for each UV radiation condition, and  $n = 108$  for each rainfall regime. Different letters indicate significant differences among seasons and/or treatment conditions. Interaction between factors were only mentioned when  $p$ -values  $\leq 0.05$ . The significance level considered was  $p \leq 0.05$ ; A, photosynthetic rate; Car, carotenoids; Chl, chlorophyll; E, transpiration rate; ETR, apparent electron transport rate;  $F_v/F_m$ , maximum photochemical efficiency of PSII; GAE, mg of gallic acid equivalents; gs, stomatal conductance; LD, leaf density; LMA, leaf mass per area; NPQ, non-photochemical quenching; RWC, relative water content; WUE, water use efficiency; ns, not significant; p, p values.

|   | UV radiation treatment (UV) |                    |                    |                    | Rainfall treatment (P) |  |                    |                    | Seasonal effect (S)  |                      |                     |                      |
|---|-----------------------------|--------------------|--------------------|--------------------|------------------------|--|--------------------|--------------------|----------------------|----------------------|---------------------|----------------------|
|   | UV0                         |                    | UVBA               |                    | p                      |  | Natural rainfall   |                    | Reduced rainfall     |                      | p                   |                      |
|   | UV0                         | UVA                | UVBA               | UVBA               | p                      |  | Natural rainfall   | Reduced rainfall   | Autumn               | Winter               | Spring              | Summer               |
| <i>A. unedo</i>   |                             |                    |                    |                    |                        |  |                    |                    |                      |                      |                     |                      |
| Area ( $\text{cm}^2$ )                                  | 10.28 $\pm$ 0.77            | 10.62 $\pm$ 0.65   | 10.60 $\pm$ 0.67   | 10.60 $\pm$ 0.67   | ns                     |  | 10.96 $\pm$ 0.45   | 10.05 $\pm$ 0.58   | 7.75 $\pm$ 0.66 a    | 10.18 $\pm$ 0.39 a   | 14.11 $\pm$ 0.67 c  | 9.97 $\pm$ 0.62 a    |
| Thickness ( $\mu\text{m}$ )                             | 398.24 $\pm$ 15.3           | 400.05 $\pm$ 15.33 | 418.81 $\pm$ 14.24 | 418.81 $\pm$ 14.24 | ns                     |  | 407.24 $\pm$ 13.05 | 404.26 $\pm$ 11.30 | 466.21 $\pm$ 8.66 a  | 470.23 $\pm$ 7.06 a  | 331.61 $\pm$ 4.38 b | 354.76 $\pm$ 12.16 b |
| LMA ( $\text{mg cm}^{-2}$ )                             | 16.10 $\pm$ 0.69            | 16.62 $\pm$ 0.78   | 16.61 $\pm$ 0.63   | 16.61 $\pm$ 0.63   | ns                     |  | 16.74 $\pm$ 0.63   | 16.15 $\pm$ 0.51   | 18.31 $\pm$ 0.59 a   | 19.98 $\pm$ 0.34 b   | 12.20 $\pm$ 0.19 c  | 15.29 $\pm$ 0.35 d   |
| LD ( $\text{mg cm}^{-3}$ )                              | 404.78 $\pm$ 7.44           | 414.75 $\pm$ 9.35  | 397.72 $\pm$ 6.54  | 397.72 $\pm$ 6.54  | ns                     |  | 411.36 $\pm$ 7.02  | 400.14 $\pm$ 5.74  | 393.10 $\pm$ 8.87 a  | 425.55 $\pm$ 5.51 b  | 368.06 $\pm$ 3.99 c | 436.29 $\pm$ 7.10 b  |
| RWC (%)   | 81.21 $\pm$ 1.42            | 79.28 $\pm$ 2.51   | 82.52 $\pm$ 1.98   | 82.52 $\pm$ 1.98   | ns                     |  | 79.86 $\pm$ 1.86   | 82.15 $\pm$ 1.39   | 85.49 $\pm$ 0.98 a   | 75.28 $\pm$ 1.13 b   | 89.99 $\pm$ 0.53 a  | 73.26 $\pm$ 2.93 b   |
| E ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ )    | 2.19 $\pm$ 0.18             | 2.08 $\pm$ 0.16    | 2.13 $\pm$ 0.18    | 2.13 $\pm$ 0.18    | ns                     |  | 1.96 $\pm$ 0.13    | 2.31 $\pm$ 0.14    | 1.94 $\pm$ 0.14 a    | 2.54 $\pm$ 0.13 b    | 2.68 $\pm$ 0.24 b   | 1.38 $\pm$ 0.09 a    |
| gs ( $\text{mmol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ )  | 95.56 $\pm$ 13.76           | 86.57 $\pm$ 10.98  | 84.13 $\pm$ 11.87  | 84.13 $\pm$ 11.87  | ns                     |  | 77.66 $\pm$ 8.74   | 99.85 $\pm$ 10.75  | 155.01 $\pm$ 14.97 a | 109.36 $\pm$ 6.86 a  | 53.76 $\pm$ 5.14 b  | 36.88 $\pm$ 2.82 b   |
| A ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ ) | 7.56 $\pm$ 0.68             | 8.05 $\pm$ 0.58    | 7.54 $\pm$ 0.65    | 7.54 $\pm$ 0.65    | ns                     |  | 6.84 $\pm$ 0.49    | 8.60 $\pm$ 0.50    | 9.66 $\pm$ 0.55 a    | 7.95 $\pm$ 0.36 a    | 8.42 $\pm$ 0.83 a   | 4.84 $\pm$ 0.60 b    |
| WUE ( $\mu\text{mol mmol}^{-1}$ )                       | 99.2 $\pm$ 12.41            | 114.3 $\pm$ 9.99   | 109.7 $\pm$ 8.67   | 109.7 $\pm$ 8.67   | ns                     |  | 107.7 $\pm$ 9.14   | 107.8 $\pm$ 7.93   | 68.2 $\pm$ 4.08 a    | 75.4 $\pm$ 3.38 a    | 159 $\pm$ 6.42 b    | 128.3 $\pm$ 14.17 b  |
| ETR   | 108.57 $\pm$ 6.62           | 101.76 $\pm$ 6.27  | 111.97 $\pm$ 6.53  | 111.97 $\pm$ 6.53  | ns                     |  | 106.69 $\pm$ 5.29  | 108.18 $\pm$ 5.31  | 103.99 $\pm$ 6.11 a  | 137.51 $\pm$ 6.31 b  | 112.23 $\pm$ 4.61 a | 76.00 $\pm$ 4.42 c   |
| $F_v/F_m$   | 0.73 $\pm$ 0.01             | 0.73 $\pm$ 0.02    | 0.74 $\pm$ 0.02    | 0.74 $\pm$ 0.02    | ns                     |  | 0.72 $\pm$ 0.02    | 0.74 $\pm$ 0.01    | 0.76 $\pm$ 0.01 a    | 0.74 $\pm$ 0.01 a    | 0.78 $\pm$ 0.004 a  | 0.65 $\pm$ 0.04 b    |
| NPQ   | 3.19 $\pm$ 0.22             | 3.19 $\pm$ 0.22    | 3.61 $\pm$ 0.24    | 3.61 $\pm$ 0.24    | ns                     |  | 3.44 $\pm$ 0.20    | 3.22 $\pm$ 0.17    | 3.38 $\pm$ 0.23 a    | 3.86 $\pm$ 0.27 a    | 3.68 $\pm$ 0.16 a   | 2.39 $\pm$ 0.25 b    |
| Chl a ( $\text{mg g}^{-1}$ DW)                          | 1.37 $\pm$ 0.11             | 1.36 $\pm$ 0.01    | 1.40 $\pm$ 0.01    | 1.40 $\pm$ 0.01    | ns                     |  | 1.35 $\pm$ 0.09    | 1.41 $\pm$ 0.07    | 1.64 $\pm$ 0.05 a    | 1.64 $\pm$ 0.05 a    | 0.40 $\pm$ 0.02     | 0.38 $\pm$ 0.017 ns  |
| Chl b ( $\text{mg g}^{-1}$ DW)                          | 0.39 $\pm$ 0.02             | 0.39 $\pm$ 0.02    | 0.40 $\pm$ 0.03    | 0.40 $\pm$ 0.03    | ns                     |  | 0.39 $\pm$ 0.02    | 0.40 $\pm$ 0.02    | 0.40 $\pm$ 0.02 ns   | 0.40 $\pm$ 0.02 ns   | 2.04 $\pm$ 0.07 a   | 1.50 $\pm$ 0.07 b    |
| Chl a + b ( $\text{mg g}^{-1}$ DW)                      | 1.75 $\pm$ 0.13             | 1.76 $\pm$ 0.11    | 1.80 $\pm$ 0.12    | 1.80 $\pm$ 0.12    | ns                     |  | 1.74 $\pm$ 0.11    | 1.81 $\pm$ 0.09    | 1.74 $\pm$ 0.11 ns   | 1.81 $\pm$ 0.09 ns   | 0.48 $\pm$ 0.01 a   | 0.39 $\pm$ 0.02 b    |
| Car ( $\text{mg g}^{-1}$ DW)                            | 0.43 $\pm$ 0.03             | 0.44 $\pm$ 0.02    | 0.43 $\pm$ 0.02    | 0.43 $\pm$ 0.02    | ns                     |  | 0.43 $\pm$ 0.02    | 0.44 $\pm$ 0.02 ns | 0.44 $\pm$ 0.02 ns   | 0.48 $\pm$ 0.01 a    | 4.13 $\pm$ 0.11 a   | 2.93 $\pm$ 0.06 b    |
| Chl a/Chl b   | 3.50 $\pm$ 0.19             | 3.46 $\pm$ 0.18    | 3.64 $\pm$ 0.25    | 3.64 $\pm$ 0.25    | ns                     |  | 3.48 $\pm$ 0.17    | 3.59 $\pm$ 0.17    | 0.25 $\pm$ 0.01 ns   | 0.24 $\pm$ 0.01 a    | 0.24 $\pm$ 0.01 a   | 0.26 $\pm$ 0.01 b    |
| Car/Chl a + b   | 0.25 $\pm$ 0.01             | 0.25 $\pm$ 0.01    | 0.25 $\pm$ 0.01    | 0.25 $\pm$ 0.01    | ns                     |  | 0.25 $\pm$ 0.01    | 0.25 $\pm$ 0.01 ns | 0.25 $\pm$ 0.01 ns   | 0.24 $\pm$ 0.01 a    | 0.24 $\pm$ 0.01 a   | 0.26 $\pm$ 0.01 b    |
| GAE ( $\text{mg g}^{-1}$ DW)                            | 248.55 $\pm$ 11.95          | 265.79 $\pm$ 8.19  | 252.58 $\pm$ 8.30  | 252.58 $\pm$ 8.30  | ns                     |  | 252.41 $\pm$ 6.18  | 258.87 $\pm$ 9.34  | 261.59 $\pm$ 9.06    | 261.59 $\pm$ 9.06    | 249.69 $\pm$ 6.34   | 249.69 $\pm$ 6.34    |
| <i>P. angustifolia</i>                                  |                             |                    |                    |                    |                        |  |                    |                    |                      |                      |                     |                      |
| Area ( $\text{cm}^2$ )                                  | 2.33 $\pm$ 0.13             | 2.20 $\pm$ 0.09    | 2.20 $\pm$ 0.12    | 2.20 $\pm$ 0.12    | ns                     |  | 2.20 $\pm$ 0.09    | 2.28 $\pm$ 0.09    | 2.05 $\pm$ 0.09 a    | 2.47 $\pm$ 0.14 ab   | 2.79 $\pm$ 0.15 b   | 1.68 $\pm$ 0.10 c    |
| Thickness ( $\mu\text{m}$ )                             | 434.05 $\pm$ 9.30           | 418.32 $\pm$ 8.71  | 427.69 $\pm$ 8.54  | 427.69 $\pm$ 8.54  | ns                     |  | 429.86 $\pm$ 6.97  | 423.23 $\pm$ 7.50  | 466.64 $\pm$ 5.95 a  | 481.67 $\pm$ 10.49 a | 340.22 $\pm$ 6.41 b | 405.44 $\pm$ 6.63 c  |
| LMA ( $\text{mg cm}^{-2}$ )                             | 15.75 $\pm$ 0.41            | 14.86 $\pm$ 0.41   | 15.61 $\pm$ 0.52   | 15.61 $\pm$ 0.52   | ns                     |  | 15.57 $\pm$ 0.39   | 15.23 $\pm$ 0.34   | 17.49 $\pm$ 0.31 a   | 18.01 $\pm$ 0.29 a   | 10.16 $\pm$ 0.25 b  | 15.29 $\pm$ 0.49 c   |
| LD ( $\text{mg cm}^{-3}$ )                              | 361.81 $\pm$ 5.68           | 353.04 $\pm$ 5.76  | 361.70 $\pm$ 8.42  | 361.70 $\pm$ 8.42  | ns                     |  | 359.93 $\pm$ 6.08  | 357.51 $\pm$ 4.76  | 374.80 $\pm$ 5.19 a  | 378.79 $\pm$ 6.08 a  | 299.26 $\pm$ 5.94 b | 377.10 $\pm$ 8.85 a  |
| RWC (%)   | 74.00 $\pm$ 0.80            | 74.21 $\pm$ 0.91   | 73.44 $\pm$ 0.95   | 73.44 $\pm$ 0.95   | ns                     |  | 74.32 $\pm$ 0.75   | 73.46 $\pm$ 0.70   | 75.18 $\pm$ 0.90 ab  | 70.79 $\pm$ 0.87 c   | 77.61 $\pm$ 0.95 a  | 71.97 $\pm$ 1.18 cb  |
| E ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ )    | 2.80 $\pm$ 0.19             | 2.54 $\pm$ 0.13    | 2.89 $\pm$ 0.14    | 2.89 $\pm$ 0.14    | ns                     |  | 2.68 $\pm$ 0.12    | 2.80 $\pm$ 0.14    | 2.63 $\pm$ 0.21 a    | 2.70 $\pm$ 0.14 a    | 3.68 $\pm$ 0.16 b   | 1.97 $\pm$ 0.11 c    |
| gs ( $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ )   | 150.34 $\pm$ 20.16          | 114.62 $\pm$ 9.01  | 122.58 $\pm$ 9.58  | 122.58 $\pm$ 9.58  | ns                     |  | 117.09 $\pm$ 8.25  | 139.90 $\pm$ 13.27 | 216.79 $\pm$ 24.20 a | 145.34 $\pm$ 9.80 b  | 96.72 $\pm$ 5.90 c  | 55.12 $\pm$ 3.40 d   |
| A ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ ) | 13.50 $\pm$ 1.17            | 12.55 $\pm$ 0.86   | 12.26 $\pm$ 0.83   | 12.26 $\pm$ 0.83   | ns                     |  | 12.20 $\pm$ 0.74   | 13.31 $\pm$ 0.81   | 18.75 $\pm$ 1.72 a   | 10.09 $\pm$ 0.51 b   | 13.73 $\pm$ 0.51 c  | 8.37 $\pm$ 0.49 b    |
| WUE ( $\mu\text{mol}/\text{mmol}^{-1}$ )                | 132.08 $\pm$ 12.46          | 138.51 $\pm$ 9.84  | 121.76 $\pm$ 7.17  | 121.76 $\pm$ 7.17  | ns                     |  | 131.99 $\pm$ 9.09  | 130.15 $\pm$ 7.22  | 108.79 $\pm$ 12.66 a | 80.18 $\pm$ 4.13 a   | 162.91 $\pm$ 8.55 b | 170.93 $\pm$ 13.57 b |
| ETR   | 110.63 $\pm$ 5.76           | 114.65 $\pm$ 4.82  | 111.06 $\pm$ 6.21  | 111.06 $\pm$ 6.21  | ns                     |  | 111.66 $\pm$ 4.52  | 112.83 $\pm$ 4.58  | 110.63 $\pm$ 6.21 a  | 141.97 $\pm$ 6.81 b  | 101.66 $\pm$ 5.79 a | 93.26 $\pm$ 4.82 a   |
| $F_v/F_m$   | 0.69 $\pm$ 0.01             | 0.70 $\pm$ 0.01    | 0.70 $\pm$ 0.01    | 0.70 $\pm$ 0.01    | ns                     |  | 0.69 $\pm$ 0.01    | 0.71 $\pm$ 0.01    | 0.69 $\pm$ 0.01 a    | 0.72 $\pm$ 0.01 b    | 0.66 $\pm$ 0.01 c   | 0.73 $\pm$ 0.01 b    |
| NPQ   | 2.26 $\pm$ 0.10             | 2.29 $\pm$ 0.10    | 2.38 $\pm$ 0.11    | 2.38 $\pm$ 0.11    | ns                     |  | 2.28 $\pm$ 0.09    | 2.34 $\pm$ 0.09    | 2.06 $\pm$ 0.10      | 2.48 $\pm$ 0.13      | 2.36 $\pm$ 0.13     | 1.79 $\pm$ 0.07 b    |
| Chl a ( $\text{mg g}^{-1}$ DW)                          | 1.85 $\pm$ 0.10             | 1.96 $\pm$ 0.11    | 1.97 $\pm$ 0.15    | 1.97 $\pm$ 0.15    | ns                     |  | 1.85 $\pm$ 0.10    | 2.01 $\pm$ 0.09    | 2.06 $\pm$ 0.10      | 2.06 $\pm$ 0.10      | 2.33 $\pm$ 0.13     | 1.79 $\pm$ 0.07 b    |
| Chl b ( $\text{mg g}^{-1}$ DW)                          | 0.51 $\pm$ 0.04             | 0.54 $\pm$ 0.03    | 0.55 $\pm$ 0.04    | 0.55 $\pm$ 0.04    | ns                     |  | 0.52 $\pm$ 0.03    | 0.54 $\pm$ 0.03    | 0.53 $\pm$ 0.03      | 0.53 $\pm$ 0.03      | 0.54 $\pm$ 0.02     | 0.54 $\pm$ 0.02      |
| Chl a + b ( $\text{mg g}^{-1}$ DW)                      | 2.37 $\pm$ 0.14             | 2.49 $\pm$ 0.14    | 2.51 $\pm$ 0.18    | 2.51 $\pm$ 0.18    | ns                     |  | 2.37 $\pm$ 0.12    | 2.55 $\pm$ 0.12    | 2.57 $\pm$ 0.12 ns   | 2.59 $\pm$ 0.14      | 2.33 $\pm$ 0.09 b   | 2.33 $\pm$ 0.09 b    |
| Car ( $\text{mg g}^{-1}$ DW)                            | 0.66 $\pm$ 0.03             | 0.67 $\pm$ 0.04    | 0.67 $\pm$ 0.04    | 0.67 $\pm$ 0.04    | ns                     |  | 0.66 $\pm$ 0.03    | 0.67 $\pm$ 0.03    | 0.67 $\pm$ 0.03 ns   | 0.76 $\pm$ 0.03 a    | 0.58 $\pm$ 0.02 b   | 0.58 $\pm$ 0.02 b    |
| Chl a/Chl b   | 3.70 $\pm$ 0.17             | 3.66 $\pm$ 0.15    | 3.60 $\pm$ 0.07    | 3.60 $\pm$ 0.07    | ns                     |  | 3.54 $\pm$ 0.09    | 3.76 $\pm$ 0.12    | 3.76 $\pm$ 0.12 ns   | 3.95 $\pm$ 0.08 a    | 3.36 $\pm$ 0.08 b   | 3.36 $\pm$ 0.08 b    |
| Car/Chl a + b   | 0.28 $\pm$ 0.01             | 0.27 $\pm$ 0.01    | 0.27 $\pm$ 0.01    | 0.27 $\pm$ 0.01    | ns                     |  | 0.28 $\pm$ 0.01    | 0.27 $\pm$ 0.01 ns | 0.27 $\pm$ 0.01 ns   | 0.30 $\pm$ 0.01      | 0.25 $\pm$ 0.01     | 0.25 $\pm$ 0.01      |
| GAE ( $\text{mg g}^{-1}$ DW)                            | 111.75 $\pm$ 2.82           | 110.27 $\pm$ 3.23  | 107.07 $\pm$ 2.61  | 107.07 $\pm$ 2.61  | ns                     |  | 112.21 $\pm$ 2.42  | 107.51 $\pm$ 2.36  | 116.18 $\pm$ 2.30 a  | 116.18 $\pm$ 2.30 a  | 103.77 $\pm$ 2.23 b | 103.77 $\pm$ 2.23 b  |



**Fig. 2.** Leaf (A) transpiration rates ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), (B) stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), (C) net photosynthetic rates ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and (D) intrinsic water use efficiency (WUE,  $\mu\text{mol mol}^{-1}$ ) of *Arbutus unedo* plants developed under three UV conditions (UV0, UVA, UVBA) and two rainfall regimes (natural and reduced rainfall). Bars represent means  $\pm$  standard error pooling data from the four seasons ( $n = 12$ ). Asterisks indicate significant differences ( $p \leq 0.05$ ) within each UV condition.

Regarding ETR, plants under reduced precipitation showed 23% higher values when grown exposed only to UV-A compared to plants exposed to near-ambient UV ( $F_{2,112} = 3.03$ ;  $p = 0.05$ ) (Fig. 3E). Moreover, ETR was 20% lower in UVBA plants grown with reduced rainfall than in those grown under natural rainfall ( $F_{1,71} = 4.53$ ;  $p = 0.04$ ) (Fig. 3E). In the case of  $F_v/F_m$ , plants grown under natural rainfall showed higher values when they were exposed to UVA and UVBA than when they were grown with almost no UV (UV0 plants), with differences being 4.0%

and 5.3%, respectively ( $F_{2,81} = 6.33$ ;  $p = 0.01$ ) (Fig. 3F). In addition, UV0 plants had 5.7% lower  $F_v/F_m$  values under natural rainfall than under drier conditions ( $F_{1,50} = 11.06$ ;  $p = 0.01$ ) (Fig. 3F).

#### 3.4. Correlations between leaf traits and leaf C and N contents for both species

Significant correlation between the studied leaf traits and leaf C and N contents differed depending on the species (Table 2). In fact, in *A. unedo*, most leaf traits did not correlate with leaf C, even when the analyses were conducted within the different UV conditions (Table 2). On the contrary, leaf N content of this species correlated positively with 8 of the 16 leaf traits measured (thickness, LMA,  $E$ ,  $g_s$ , ETR and the content of Chl *a* and Car). When the analyses were conducted within each UV condition, 7 leaf traits were still positively correlated with leaf N content in UVA plants, while only 2 and 1 significant correlations were found in UV0 and UVBA plants, respectively (Table 2).

In *P. angustifolia*, leaf C and N contents were strongly correlated with many of the leaf traits measured (area, thickness, LMA,  $E$ ,  $g_s$ , A, ETR and the content of Car, and also of Chl *a* for leaf N), being the correlations negative in the case of leaf C and positive in the case of leaf N (Table 2). Similar significant correlations were found when the analyses were performed within each UV condition (Table 2).

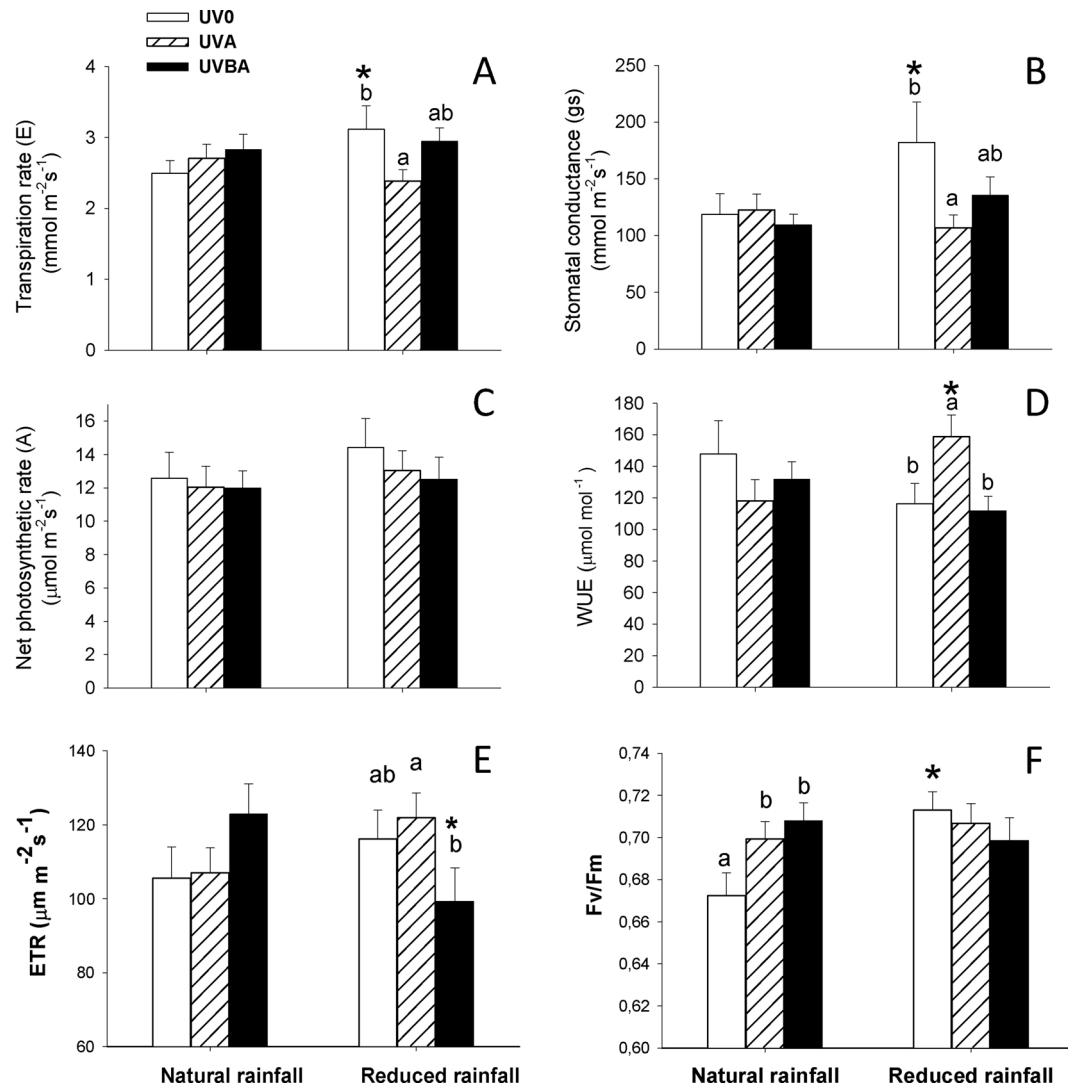
#### 3.5. Seasonal effects on *Arbutus unedo* L. and *Phillyrea angustifolia* L. leaf traits

Seasonality exerted a strong effect on the morphological, physiological and biochemical parameters analysed in leaves of *A. unedo* and *P. angustifolia*, independently of the treatments applied, showing both species similar seasonal fluctuations (Table 1). With regard to leaf morphological traits, *A. unedo* and *P. angustifolia* had thicker leaves in autumn and winter than in spring and summer (Table 1). LMA followed the same trend as leaf thickness, despite leaf area was more variable in both species, with the biggest leaves being detected in the spring sampling. Leaf density, in both species, was significantly lower in spring than in the rest of the seasons.

Considering physiological parameters (Table 1), leaves of both species had higher RWC in spring and autumn, the most humid seasons in the Mediterranean climate region, than in winter and summer. In accordance with this, the highest  $E$  and  $g_s$  of both species were recorded in spring and autumn, respectively, while the lowest values of these parameters were found in summer. Indeed,  $g_s$  of *A. unedo* and *P. angustifolia* leaves were four folds higher in autumn than in summer. Maximum and minimum A values were also recorded in both species in autumn and summer, respectively, although, for *A. unedo*, differences were not significant among autumn, winter or spring seasons. Leaf WUE of both species was lower in autumn and winter than in spring and summer (Table 1).

With regard to chlorophyll fluorescence parameters (Table 1), the highest leaf ETR of both species was found in winter and the lowest in summer, although for *P. angustifolia* differences were not significant among autumn, spring or summer seasons. In *A. unedo*, the lowest values of leaf  $F_v/F_m$  and NPQ were also recorded in summer. Strikingly, in the case of *P. angustifolia*, the lowest values of  $F_v/F_m$  were found in spring and autumn, while no significant differences among seasons were recorded for NPQ.

The leaf content of Chl *a* and Car, as well as the Chl *a*/Chl *b* ratio, were greater in winter than in summer in both species (Table 1). But, differences in the leaf content of Chl *a* + *b* between winter and summer samplings was only significant in *A. unedo* (26%), which could explain the lower leaf Car/Chl ratio observed in *A. unedo* in winter compared to summer and the opposite in *P. angustifolia*. Further, higher leaf content of Car in winter than in summer in *A. unedo*, were only significant in plants growing without UV-B (52% and 10% higher in UV0 and UVA plants, respectively), which would explain the interactive effect found



**Fig. 3.** Leaf (A) transpiration rates ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), (B) stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), (C) net photosynthetic rates ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), (D) intrinsic water use efficiency (WUE,  $\mu\text{mol mmol}^{-1}$ ), (E) apparent electron transport rates (ETR,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and (F) maximum photochemical efficiency of PSII ( $F_v/F_m$ ) of *Phillyrea angustifolia* plants developed under three UV conditions (UV0, UVA, UVBA) and two rainfall regimes (natural and reduced rainfall). Bars represent means  $\pm$  standard errors ( $n = 36$ , data from the four seasons was pooled). Different lower-case letters indicate significant differences ( $P \leq 0.05$ ) within each rainfall regime, whereas asterisks indicate significant differences ( $P \leq 0.05$ ) within each UV condition.

( $F_{2,12} = 5.86$ ,  $p = 0.02$ ; Table 1). The leaf content of phenolic compounds from both species was also higher in winter than in summer, although differences were only statistically significant for *P. angustifolia* (Table 1).

## 4. Discussion

### 4.1. UV or rainfall treatment effects

In general, the evergreen Mediterranean shrubs *A. unedo* and *P. angustifolia* were not very sensitive to the applied UV exclusion or rainfall reduction, since only a few parameters responded to the treatments. In particular, leaf gas exchange rates of *A. unedo* plants changed in response to drier conditions while leaf gas exchange rates, ETR and  $F_v/F_m$  of *P. angustifolia* plants responded to the combination of both treatments (Table 1).

Contrary to what it was expected, morphological leaf traits were responsive to the treatments in any of the two species. In general, it is widely accepted that plants tend to have smaller and thicker leaves with higher LMA in response to UV-B (Jansen and Greenberg, 1998), as it was observed for several Mediterranean species grown in a greenhouse,

among them *P. angustifolia* (Verdaguer et al., 2012; Bernal et al., 2013). In the current study, the lack of response of *P. angustifolia* at morphological level could be partly attributed to the differences between the experimental setups, mainly in relation to the age and growth conditions of plants. In fact, leaves from naturally growing *P. angustifolia* plants from this experiment were on average 31% smaller, 37% thicker and with an index of sclerophylly (LMA) 15% higher than leaves from the one-year-old seedlings used in the previous study (Verdaguer et al., 2012). Taking into account that the leaf sclerophylly index has been positively associated with the species tolerance to UV-B radiation (Verdaguer et al., 2012), the higher LMA found in leaves from wild *P. angustifolia* plants might explain the lack of response to UV radiation at the morphological level. *A. unedo* leaves showed similar LMA values to those found for *P. angustifolia*, which were also higher (by 17%) than those reported for potted seedlings of this species (Díaz-Guerra, 2017); so, it is not surprising that UV did not affect either the leaf morphology of this species. These results underpin the modulation of plant UV-responses by plant life-history (Kuhlmann and Müller, 2009), and, thus, despite some approaches (such as controlled experiments using seedlings) allow to delve deeper into the mechanisms of plant responses to environmental factors, information should be used with caution when



**Table 2**  
Pearson's correlations between leaf morphological, physiological and biochemical traits and the leaf carbon and nitrogen content ( $\text{mg g}^{-1}$  DW) and within each UV condition for *A. unedo* and *P. angustifolia* plants. In the case of *A. unedo*, N = 24 for Area, Thickness, LMA, LD, RWC, E, g<sub>s</sub>, A, ETR, F<sub>v</sub>/F<sub>m</sub> and NPQ. N = 12 for Chla, Chlb, Carotenoid, LMA, LD, RWC, E, g<sub>s</sub>, A, ETR, F<sub>v</sub>/F<sub>m</sub> and NPQ. N = 36 for Chla, Chlb, Carotenoid, GAE, C and N.

|  | <i>A. unedo</i> |          |                        |          | <i>P. angustifolia</i> |    |                        |    | <i>P. angustifolia</i> |    |                        |    |
|--|-----------------|----------|------------------------|----------|------------------------|----|------------------------|----|------------------------|----|------------------------|----|
|  | <i>A. unedo</i> |          | <i>P. angustifolia</i> |          | <i>A. unedo</i>        |    | <i>P. angustifolia</i> |    | <i>A. unedo</i>        |    | <i>P. angustifolia</i> |    |
|  | C               | N        | C                      | N        | C                      | N  | C                      | N  | C                      | N  | C                      | N  |
| Area ( $\text{mm}^2$ )   | ns              | ns       | –0.689***              | 0.816*** | ns                     | ns | –0.792**               | ns | –0.792**               | ns | –0.792**               | ns |
| Thickness (mm)   | ns              | 0.542**  | –0.705***              | 0.677*** | ns                     | ns | –0.787**               | ns | –0.787**               | ns | –0.787**               | ns |
| LMA ( $\text{mg mm}^{-2}$ )                                    | ns              | 0.510*   | –0.588***              | 0.558*** | ns                     | ns | –0.721**               | ns | –0.721**               | ns | –0.721**               | ns |
| LD ( $\text{mg mm}^{-3}$ )                                     | ns              | ns       | ns                     | ns       | ns                     | ns | ns                     | ns | ns                     | ns | ns                     | ns |
| RWC (%)  | ns              | ns       | ns                     | ns       | ns                     | ns | ns                     | ns | ns                     | ns | ns                     | ns |
| E ( $\text{mmH}_2\text{O m}^{-2} \text{s}^{-1}$ )              | –0.394*         | 0.433**  | –0.518**               | 0.586*** | ns                     | ns | –0.804**               | ns | –0.804**               | ns | –0.804**               | ns |
| g <sub>s</sub> ( $\text{mmH}_2\text{O m}^{-2} \text{s}^{-1}$ ) | –0.478**        | 0.481**  | –0.688***              | 0.759*** | ns                     | ns | –0.697*                | ns | –0.697*                | ns | –0.697*                | ns |
| A ( $\mu\text{mCO}_2 \text{m}^{-2} \text{s}^{-1}$ )            | ns              | ns       | –0.523**               | 0.437**  | ns                     | ns | –0.797**               | ns | –0.797**               | ns | –0.797**               | ns |
| ETR  | ns              | 0.725*** | –0.588***              | 0.630*** | ns                     | ns | 0.756**                | ns | 0.756**                | ns | 0.756**                | ns |
| F <sub>v</sub> /F <sub>m</sub>                                 | ns              | ns       | ns                     | ns       | ns                     | ns | ns                     | ns | ns                     | ns | ns                     | ns |
| NPQ  | ns              | 0.375*   | ns                     | ns       | ns                     | ns | ns                     | ns | ns                     | ns | ns                     | ns |
| Chla ( $\text{mg g}^{-1}$ DW)                                  | ns              | 0.632*** | ns                     | 0.447*** | ns                     | ns | 0.863***               | ns | 0.863***               | ns | 0.863***               | ns |
| Chlb ( $\text{mg g}^{-1}$ DW)                                  | ns              | ns       | ns                     | ns       | ns                     | ns | 0.691*                 | ns | 0.691*                 | ns | 0.691*                 | ns |
| Car ( $\text{mg g}^{-1}$ DW)                                   | ns              | 0.600*** | –0.456**               | 0.574*** | ns                     | ns | 0.798**                | ns | 0.798**                | ns | 0.798**                | ns |
| GAE ( $\text{mg g}^{-1}$ DW)                                   | ns              | ns       | ns                     | ns       | ns                     | ns | ns                     | ns | ns                     | ns | ns                     | ns |
| N ( $\text{mg g}^{-1}$ DW)                                     | ns              | ns       | –0.701***              | ns       | ns                     | ns | –0.751**               | ns | –0.751**               | ns | –0.751**               | ns |

A, photosynthetic rate; C, carbon content; Car, carotenoids; Chl, chlorophyll; E, transpiration rate; ETR, apparent electron transport rate; F<sub>v</sub>/F<sub>m</sub>, maximum photochemical efficiency of PSII; GAE, mg of gallic acid equivalents; g<sub>s</sub>, stomatal conductance; LD, leaf density; LMA, leaf mass per area; N, Nitrogen content; NPQ, non-photochemical quenching; RWC, relative water content; WUE, intrinsic water use efficiency; ns, not significant; asterisks indicate significant correlations with  $p \leq 0.001$  (\*\*\*),  $p \leq 0.01$  (\*\*) and  $p \leq 0.025$  (\*).

scaling to natural plant communities (Kunz et al., 2016).

*A. unedo* leaves were very rich in phenolic compounds, having about 250 mg AG g<sup>-1</sup> DW, 2-fold more than *P. angustifolia* leaves, and more than most Mediterranean woody species grown at similar latitudes, even than co-occurring species (Bernal et al., 2013; Grifoni et al., 2016). Phenols are associated with multiple functions in plants. Particularly, they can act as UV-screening and/or as antioxidants in response to UV (Julkunen-Tiitto et al., 2005; Agati and Tattini, 2010). Despite the content of total phenols did not vary in *A. unedo* leaves in response to UV-B, in a parallel study in which the leaf phenolic profile of these plants was described, we found a UV-B-induced increase in the content of quercetins, which have antioxidant properties (Nenadis et al., 2015). Hence, the general UV-tolerance shown by *A. unedo* plants could be linked not only to their high degree of leaf sclerophylly, but also to their elevated total content of phenols, in agreement with what it has been previously reported for some Mediterranean species (Bussotti et al., 2013).

Strikingly, whereas the leaf C content of *A. unedo* did not correlate with any leaf trait under any of the different UV conditions, strong correlations were found between the leaf morpho-physiological and biochemical traits and the leaf N content in those plants growing exposed only to UV-A (Table 2). Indeed, in UV-A plants of this species, the higher LMA, *E*, *g<sub>s</sub>*, and Chl (*a* and *b*) and Car amounts, the higher the leaf N content was. Taking into account that leaf parameters that correlated with leaf N content did not respond to the UV treatment, nor the N content (Díaz-Guerra et al., in press), the correlations found could indicate some type of close tie and specific N metabolism regulation promoted by UV-A, resulting in a more efficient plant development and N consumption. A previous study by Tezuka et al. (1994) found that, in *Raphanus sativus* seedlings, root N uptake and leaf concentration of soluble proteins increased in plants under UV-A. Krywult et al. (2008) also reported an inhibition of the nitrate reductase activity in Scots pine in response to the exclusion of UV-A. However, in some *Glycine max* varieties, N metabolism was not affected by UV-A exposure (Baroniya et al., 2014), which could be attributed to differences in experimental setups or UV-A-species-specific responses. Indeed, in *P. angustifolia*, the number of leaf parameters that correlated with leaf N content was similar among UV conditions (Table 2). Hence, more studies are needed to clarify the possible role of UV-A in the N metabolism regulation of some species.

High values of leaf sclerophylly and phenolic content have also been related to a higher plant resistance to drought (Bussotti et al., 2013; Selmar and Kleinwächter, 2013). In the present study, although the soil water content was significantly decreased in plots receiving reduced rainfall (by 20%) (Fig. S1), the leaf RWC of *A. unedo* plants did not respond to the UV and/or rainfall treatments. In fact, *A. unedo* plants grown under a reduction of precipitation had the highest values of *E*, *g<sub>s</sub>* and *A* (Table 1). One possible explanation of these results could be that *A. unedo* have mechanisms to cope with the experimental soil water decrease, maintaining the plant with an adequate water status, which would support an increase of stomatal conductance and photosynthetic rates, despite rising also water loss through transpiration. These results are in agreement with the water-spender strategy described for *A. unedo* to cope with drought (Fusaro et al., 2013; Sperlich et al., 2015). Higher transpiration rates due to higher stomatal conductance under reduced rainfall could be explained by the fact that *A. unedo* have a dimorphic root system with shallow and deep growing roots that enable plants to regulate the amount of groundwater uptake by deep roots when decreasing soil moisture (Filella and Peñuelas, 2003; Alessio et al., 2004; Barbata et al., 2015).

Nevertheless, it is worth noting that while *A. unedo* plants showed a reduction in *E*, *g<sub>s</sub>* and *A* (Table 1) in summer compared to the rest of seasons, which is in consonance with previous results also obtained in natural growing plants (Martínez-Vilalta et al., 2003; Mereu et al., 2009), these parameters (*E*, *g<sub>s</sub>* and *A*) were improved in plants growing under the experimental rainfall reduction (Table 1). According to

Mereu et al. (2009), *A. unedo* would have a high capacity to acclimate to drier conditions, diminishing, for instances, their leaf area/sap flow area ratio to increase available water per unit leaf area. Hence, taking into account that the present study comprises a long-term experiment, results obtained could also be explained by drought induced acclimation, becoming plants under reduced rainfall less sensitive to the adverse conditions. However, a more complete study including stem and root morphology and also root distribution, root absorbing surface and root permeability would be needed.

#### 4.2. Interactive effects between UV and rainfall treatments

In *P. angustifolia*, as in *A. unedo*, leaf RWC did not vary according to the rainfall treatment (Table 1). However, only plants growing under reduced rainfall were sensitive to UV radiation, as it was observed in other Mediterranean species (Bernal et al., 2013; Bernal et al., 2015). In particular, plants of *P. angustifolia* under low water availability had lower *E* and *g<sub>s</sub>* values when growing in UVA plots than in UV0 ones, whereas these values did not differ from control plants (UVBA plots) (Fig. 3A and B). In UVBA plants, *g<sub>s</sub>* and *E* were not affected by the rainfall regime (Fig. 3A and B). Previous studies have reported that *P. angustifolia* copes with hydric stress displaying a water-saver strategy, closing stomata to reduce water loss, increasing water use efficiency (Peñuelas et al., 2004; Vitale et al., 2012; Altieri et al., 2015). Hence, current results suggest that *P. angustifolia* plants growing under near-ambient UV radiation and reduced rainfall would not have triggered the typical conservative drought response described for this species in previous studies. The lack of response could be attributed to the fact that leaf RWC did not diminish in plants under reduced rainfall, while in the study of Peñuelas et al. (2004) a severe drought stress was applied and the leaf RWC of *P. angustifolia* plants decreased by 50% compared to controls. Otherwise, our results suggest that, under a mild water deficit, UV-A would prevent the stomatal opening observed in plants grown with almost no UV, promoting an increase in WUE (Fig. 3B and D). Since leaf *A* did not respond to the treatments, this increase in WUE (*A/g<sub>s</sub>*) would be mainly caused by the reduction in stomatal conductance. Hence, these results suggest that UV-A could trigger the water-saver response described for *P. angustifolia* under water shortage (Vitale et al., 2012). This would be supported by the fact that plants under reduced rainfall but not exposed to UV (UV0) had higher *E*, *g<sub>s</sub>* and *F<sub>v</sub>/F<sub>m</sub>* values than plants receiving all the rainfall (Fig. 3A, B, F), which suggests that the lack of UV-A would have cancelled the water-saver response of this species to the 20% decrease in soil water content. Our results on WUE also suggest that plant exposure to UV-B would counteract this UV-A-mediated response to a mild water deficit (Fig. 3D). Under drier conditions, the reduction in WUE found in plants of UVAB plots compared to those grown exposed only to UV-A alone would be in agreement with their lower ETR values (Fig. 3E).

*A. unedo* plants were basically tolerant to UV, but plants growing exposed only to UV-A showed higher *g<sub>s</sub>*, *E* and *A* under reduced rainfall than when they received all rainwater (Fig. 2A–C). Therefore, also in *A. unedo*, UV-A exposure would intensify the water spending response of plants to water deficit. In concordance with this, *Laurus nobilis*, also a typical water-spender species in response to drought (Nardini et al., 1996), had under low-watered UV-A-supplemented conditions higher *g<sub>s</sub>* and *A* than well-watered plants, despite the leaf RWC between well- and low-watered only decrease a 3.4% (Bernal et al., 2015). Therefore, the combined response of *P. angustifolia* and *A. unedo* (and *L. nobilis*) to a mild water shortage in conjunction with UV-A exposure would be consistent with the water use response of each species, saver or spender, shown by plants under a more severe water stress. Nevertheless, these results highlight the need of disentangling the role of UV-A (and UV-B) in plant responses to soil water shortage.

### 4.3. Seasonal effects

The effect of seasonality in perennial species involves ontogenic changes associated to plant growth and plant responses to environmental cues (Mason et al., 2013). In this study, ontogenic changes in *A. unedo* and *P. angustifolia* leaves are obvious, being mainly associated to the observed variations in leaf morphology (Table 1). For instance, leaves collected in autumn and winter were thicker and denser and had higher LMA than those collected in spring, when plants encountered the most favourable environmental conditions for growing and leaves were younger and tender (Ogaya and Penuelas, 2004; Parra et al., 2012). The increase in LMA during leaf ontogeny has been reported in various woody species, usually linked to a thickening of the cell walls and lignin accumulation, increasing leaf density and decreasing leaf *N* content in a mass basis (Wright et al., 2004 and references therein). Nevertheless, in this study, LMA and leaf thickness were positively correlated with leaf *N* content in *A. unedo* and *P. angustifolia*, being also negatively correlated with leaf *C* content in *P. angustifolia* (Table 2). Given that an increase in leaf *N* has been associated with an increase of leaf mesophyll in relation to vascular plus sclerenchymatic tissue (De La Riva et al., 2016), our results suggest that the increase in LMA could be explained by an increase in the photosynthetic machinery, which could agree with an enhanced amount of Chl *a* (Table 2). This also agrees with the higher ETR values found in the leaves of both species sampled in winter, together with their lower leaf *C/N* values ( $36.15 \pm 0.9$ ,  $39.65 \pm 1.0$  for *A. unedo* and *P. angustifolia*, respectively) compared to those of summer leaves ( $44.35 \pm 0.9$ ,  $50.55 \pm 1.4$  for *A. unedo* and *P. angustifolia*, respectively) (Díaz-Guerra et al., in press). In Mediterranean oak species, Niinemets et al. (2004) also found an increase in LMA and larger or constant *N* content, but a decrease in ETR, concomitantly with leaf aging. They attributed the reduction in ETR to a larger fraction of *N* bound to cell walls instead of being invested in photosynthetic machinery as leaves get older. In our study, the age difference among sampled leaves would be maximum one year, which would likely not be enough to find a shift to a leaf resource-conservative strategy (Ishida et al., 2005). In *P. angustifolia*, strong correlations between leaf traits and *N* and *C* contents were found. On the contrary, in *A. unedo*, leaf traits were mainly correlated with *N* but not with *C* (Table 2), which might be attributable to a high investment of carbon in the leaf phenolic pool, since this species showed a huge amount of foliar phenols in comparison to other Mediterranean woody species (Bernal et al., 2013; Grifoni et al., 2016).

The seasonal variation of the physiological parameters studied in both species was consistent with the environmental fluctuations inherent to the Mediterranean climate. As expected, the lowest leaf RWC values were found in samples of both species collected in summer and winter, i.e. the drier seasons, with leaf *g<sub>s</sub>* and *E* values being lower in summer than in winter (Table 1). In summer, both species showed a tight regulation of stomatal aperture decreasing *A*, but increasing WUE (Table 1). However, while summer leaves of *A. unedo* had the lowest values of ETR and *F<sub>v</sub>/F<sub>m</sub>*, ETR values of *P. angustifolia* did not differ among summer, spring and autumn (Table 1). Hence, *P. angustifolia* seems to be more tolerant to summer climatic conditions than *A. unedo*, in accordance with previous studies comparing *P. angustifolia* with other co-occurring Mediterranean shrubs (Altieri et al., 2015).

Winter *A* and *F<sub>v</sub>/F<sub>m</sub>* values of *A. unedo* leaves did not differ significantly from those obtained in autumn or spring, when air temperature and water availability were the most propitious for Mediterranean vegetative plant growth. Furthermore, ETR values of this species were higher in winter than in the other seasons, which suggests that winter environmental conditions, at least those encountered in the sampling days, did not limit *A. unedo* photosynthetic activity. These results fit well with the lower leaf *C/N* content encountered in winter leaves (Díaz-Guerra et al., in press). Further, it is notorious that the leaf content of flavonols, specifically of quercetins, was higher in winter compared to summer (Nenadis et al., 2015).

Quercetin derivatives present a high antioxidant efficiency (Agati et al., 2012), which could benefit plants susceptible to experience oxidative stress during winter days when high irradiance and low temperatures often occur (Fig. 1A). Results obtained for *P. angustifolia* were similar to those for *A. unedo*, although, in *P. angustifolia*, leaf *A* was lower in winter and summer than in autumn or spring (Table 1). In accordance with previous studies (Vitale et al., 2012), *P. angustifolia* summer stomatal closure would mainly account for *A* reduction (Table 1) (Vitale et al., 2012). Nevertheless, a reduction of photochemical efficiency (*F<sub>v</sub>/F<sub>m</sub>*) in winter to avoid irreversible damage to the photosynthetic system was reported by Vitale et al. (2012), while, in the current study, *P. angustifolia* leaves sampled in winter, despite lowering leaf *A*, showed the highest ETR and *F<sub>v</sub>/F<sub>m</sub>* values (although values of the latter parameter were similar in winter and summer). Probably, differences found with the study of Vitale et al. (2012) are related to the fact that, *P. angustifolia* seedlings grew in pots under a controlled environment, while the present study was conducted using adult plants grown under natural conditions. These plants were presumably provided by a well-developed root system and they were morphologically and physiologically adapted to the typical seasonal fluctuations of the Mediterranean climate, which could have allowed them to avoid photoinhibition in winter. Overall, comparing the physiological data obtained for both species in the different seasonal samplings, our results suggest that *A. unedo* would be more sensitive to summer adverse climatic conditions than *P. angustifolia*.

### 5. Conclusions

Throughout the study period, the ontogenic changes experienced by *A. unedo* and *P. angustifolia* leaves would involve an increase in LMA and thickness, possibly due to an increase in the photosynthetic machinery, as suggested by the positive correlation between these traits and leaf *N* content in both species. Moreover, despite seasonal changes in the studied leaf physiological traits were similar for both species, our results suggest that *A. unedo* was more sensitive than *P. angustifolia* to the summer climatic conditions. Indeed, *A. unedo* showed the lowest *A*, ETR and *F<sub>v</sub>/F<sub>m</sub>* values in summer. Nevertheless, the morphological, physiological or biochemical seasonal patterns found in the parameters analysed would have not affected the response of both species to the UV and/or rainfall treatments applied.

Regarding the UV treatment, our findings indicate that mature plants of both species grown in the field are basically tolerant to the ambient levels of UV-B and UV-A. This could partly be attributed to their elevated leaf index of sclerophylly, higher than those observed in previous studies conducted with seedlings, or as it could be the case of *A. unedo*, to their high foliar content of phenols, since phenols have been associated to UV-screening and ROS-scavenging functions. In fact, *A. unedo* respond to the experimental rainfall reduction, displaying a water-spender strategy (i.e. increased *g<sub>s</sub>*, *E* and *A*). Therefore, in response to a mild reduction of precipitation in a future scenario of climate change, *A. unedo* plants might respond spending more water, which could aggravate drought conditions at the community level.

In contrast, *P. angustifolia* plants responded to the combination of UV and rainfall treatments. In particular, plants grown under UV-A exposure were sensitive to reduced rainfall, displaying, unlike *A. unedo*, a water saving strategy, increasing WUE. Given that this response was not triggered in plants grown with almost no UV (UV0), our results suggest that UV-A would decrease the sensitivity threshold of *P. angustifolia* plants to water shortage, with UV-B masking this effect under natural conditions. In accordance, the water spending strategy showed by *A. unedo* plants under reduced rainfall would be enhanced under UV-A exposure. Hence, a mild water shortage combined with UV-A exposure would trigger the water use response (saver or spender) previously reported for each species under more severe water stress conditions. Yet, many more studies are needed to disentangle the role of UV-A in plant functioning and how this role can be modulated by water shortage.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.envexpbot.2017.12.007>.

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